EXHIBIT 26

The Transport of Carbon Particles in the Human Female Reproductive Tract

G. E. Egli, M.D., and Michael Newton, M.D.

THE METHOD by which spermatozoa reach the oviduct remains an important problem in mammalian reproduction. Since spermatozoa possess motility, it has been widely assumed to be the most important factor. However, work in cows suggests that it may not be the chief means of transport. Thus, Vandemark and Moeller recovered spermatozoa from the oviduct 2½ min. after mating. This is far sooner than could be expected on the basis of the inherent motility and sense of direction of spermatozoa.

Work in animals indicates that muscular contractions of the reproductive tract may aid in the transport of spermatozoa and that the oxytocic hormone may play a part in this process. Vandemark and Hays[11] noted that a crescendo of uterine contractions took place before and during copulation in the cow. Furthermore, stimulation of the cow's genitalia produced a rise in intramammary pressure.[7] Normally such a change is brought about by the release of oxytocin from the posterior pituitary gland during the letdown or ejection reflex as the calf or milking machine is applied to the teat.[5] Finally, in-vitro studies by Vandemark and Hays[12] demonstrated that when oxytocin was added to the solution perfusing the isolated cow's uterus, the rate of transport of spermatozoa was increased.

Evidence that the same process occurs in humans is scanty. Because of the difficulty of using spermatozoa, inert particles have occasionally been employed experimentally. Amersbach placed a cap containing a suspension of carbon particles over the cervix. Following coitus he was able to recover

From the Department of Obstetrics and Gynecology, University of Mississippi School of Medicine, Jackson, Miss.

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particles from the cervical canal. Trapl had a patient insert carmine particles into the vagina immediately after intercourse. Twenty-four hr. later at laparotomy he found numerous particles in the uterine tubes. Furthermore, it has been suggested that there may be a sucking effect as a result of uterine contractions occurring at orgasm that pulls semen through the cervix into the uterus.[8] There is also some evidence that oxytocin is released at the time of orgasm in humans.[4, 9] However, the time relationships and precise mechanisms of transport of inert particles or spermatozoa have not been elucidated in humans. The paucity of information in this regard has been pointed out by Hartman in his excellent review article.

If human spermatozoa move at a rate of 3 mm./min.,[3] it should take a spermatozoon, moving on a direct course, at least 45 min., in the average woman, to travel from the cervix to the junction of the middle and outer thirds of the tube, where fertilization occurs. If the action of the uterine or other muscles of the reproductive tract is important in humans, then not only spermatozoa but also inert particles should reach the tube much sooner than this. The present study was designed to determine whether, under reasonably controlled conditions, carbon particles could be transported quickly from the vagina to the tubes.

METHODS

It seemed desirable to set up, as far as possible, conditions that were optimal for rapid transport. Thus, patients were selected who required an elective abdominal hysterectomy that could be scheduled at or near the day of ovulation. They had to be of reproductive age, to have proved fertility, and to have relatively normal reproductive organs. A suspension of carbon particles in Dextran was made up so that the particles were similar in size to spermatozoa and that the solution was of the consistency of seminal fluid. This was done by mixing 30% Dextran with 4% bone black. In addition, it was decided to use intramuscular oxytocin to aid in the transport of the particles, because of the experimental evidence indicating its possible importance.

Three women fulfilling the above criteria were studied. In each instance the procedure was as follows: Soon after general anesthesia had been induced, the patient was placed in the lithotomy position with her head tilted downward at an angle of 15° from the horizontal. A speculum was introduced into the vagina, and 3–4 ml. of sterile carbon particles–Dextran suspension were deposited in the posterior fornix. At the same time 1 ml.

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(10 U.) of oxytocin was given intramuscularly. The speculum was removed, and the patient was immediately returned to the supine flat position. Her abdomen was promptly opened, and before the uterus was manipulated, a suture was placed tightly around the tubes about 1 cm. lateral to the uterus. The tubes were excised and taken to the laboratory, where they were flushed with saline from the infundibular portion downward. The solution was collected on clean slides and examined under the microscope for carbon particles.

RESULTS

The first patient was 32 yr. of age, gravida 6, para 6, and was at the fourteenth day of her cycle, which was usually about 28 days in length. Twenty-eight min. after the carbon-particle suspension had been deposited in the posterior fornix, the tubes were ligated and then excised. Many carbon particles were found in the washings from both tubes. On microscopic examination the endometrium was described as being early progestational.

The second patient was 30 yr. of age, gravida 6, para 6, and was at the twelfth day of her cycle, which was usually about 28 days in length. Thirty-four min. after the carbon-particle suspension had been deposited in the posterior fornix, the tubes were ligated and then excised. Carbon particles were recovered from both tubes. On microscopic examination the endometrium was described as being estrogenic.

The third patient was 41 yr. of age, gravida 8, para 7, aborta 1, and was at the thirteenth day of her cycle, which was usually about 28 days in length. She was a diabetic and had aborted three mo. previously. Twenty min. after the carbon-particle suspension had been deposited in the posterior fornix, the tubes were ligated and then excised. No carbon particles were found in the washings from either tube. On microscopic examination the endometrium was described as being early progestational.

DISCUSSION

This study indicates that in two cases, under the conditions outlined, inert carbon particles, placed in the posterior fornix of the vagina, were found 28 and 34 min. later in both tubes. How they reached the tubes is a difficult question to answer. Certainly they did not proceed by their own movements. It is reasonable to suppose that some sort of movement of the uterus and/or tubes contributed to the transport of the particles.

Movements of the reproductive organs and particularly the uterus could be due to inherent motility, general body movements, the effect of anes-

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thesia, or the influence of the injected oxytocin. The uterus undoubtedly possesses inherent motility. Conceivably this could be sufficient to aid the transport of particles into the tubes, although it might well have been decreased by the anesthesia used. Bodily movements were held to a minimum. The patients were on their backs at all times, and so virtually no opportunity for the suspension to enter the uterus or tubes by gravity was afforded. Manipulation consisted only of removing the speculum, returning the patient to the supine position, opening the abdomen, and ligating the tubes. The effect of anesthesia would be, in general, to reduce uterine motility: However, spasm of the cervix or uterotubal opening could have been relaxed by the anesthesia. The theory that oxytocin does contribute to the transport of particles is most attractive, but at the present time we have no proof of it. Further in-vivo and in-vitro experiments are being done in pursuit of a solution to this problem.

The fact that in one case transport of carbon particles to the tubes was not demonstrated is not surprising. One of several factors may have contributed to this. Possibly the hormonal conditions present in the uterus were not optimal.[2] The patient's recent abortion may have been important. Finally, it is conceivable that insufficient time was allowed for transport.

SUMMARY AND CONCLUSIONS

Carbon particles, suspended in 30% Dextran, were placed in the vagina in three anesthetized women who were about to undergo elective abdominal hysterectomy at about the time of ovulation. At the same time oxytocin was injected intramuscularly. In two of the three women carbon particles were recovered from the tubes 28 and 34 min. later.

These data, together with other work in animals and humans, support the belief that the motility of spermatozoa is not the chief factor in sperm transport. Contractions of the muscle of the uterus or other reproductive organs may be very important, and it is possible that oxytocin may play a part in this process.

The University of Mississippi Medical Center
Jackson, Miss.

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